FACTORS AFFECTING GROWTH OR SURVIVAL OF *AEROMONAS HYDROPHILA* IN FOODS

ABSTRACT

Recent surveys of retail fresh foods (fish and seafood, poultry, red meat, raw milk and vegetables) have indicated the presence of organisms of the Aeromonas hydrophila *group in virtually every sample examined. As part of these surveys,* A. hydrophila *was observed to increase in number during storage of the food at 5°C. Thus, other measures to control the growth of these organisms in retail fresh foods must be sought and evaluated; in addition, measures to destroy any* A. hydrophila *introduced into the foods must be investigated. This review discusses the various measures besides temperature (pH, NaCl) to control the growth of* A. hydrophila *in foods as well as their destruction by heat, irradiation, and sanitizers.*

INTRODUCTION

During the past several years there has been increasing interest in *Aeromonas hydrophila* and the other motile aeromonads (*A. sobria* and *A. caviae*) since isolates of these species are putative causes of foodborne gastroenteritis (Buchanan and Palumbo 1985). Concern is also increased by recent reports that *Aeromonas* is readily isolated from a variety of retail-level foods (Palumbo *et al.* 1985a; Callister and Agger 1987; Stern *et al.* 1987). Further, the microorganism occurs widely in nature, especially in the water supply. Because the organism is ubiquitious, there is a need for the identification and development of effective means for eliminating or controlling the microorganism in food products. The objectives of this article are to review the available information on controlling or eliminating *Aeromonas hydrophila* in food systems.

Temperature

Until recently, refrigeration (holding of foods at 5 °C) was depended upon to keep food safe from foodborne bacterial hazards. However, many of the recently identified foodborne pathogens, including *A. hydrophila*, can grow at 5 °C (Palumbo 1986). Palumbo *et al.* (1985b) observed ready growth of clinical isolates of *A. hydrophila* in Brain Heart Infusion broth at 4 °C (Fig. 1) and in foods held at 5 °C, Palumbo *et al.* (1985a) (Table 1). The growth of clinical isolates at 4 °C was unexpected since they were isolated as the apparent(sole) cause of human illness and a food-food poisoning syndrome link has not yet been demonstrated for *A. hydrophila* (Morgan *et al.* 1985). Growth occurred in the foods and could be detected despite the presence of large numbers of competing organisms. In many of the foods examined, the total aerobic plate count (APC) was 10- to 1000-fold higher than the *A. hydrophila*. Thus, while *A. hydrophila* competed only to a limited extent in these foods, it did increase in number, and in one poultry sample did comprise *ca* 10% of the APC (Palumbo *et al.* 1985a). Callister and Agger (1987) detected *Aeromonas* sp. in retail vegetables and observed increases in number during storage at 5 °C (Table 2). The growth in vegetables during refrigerated storage was not as extensive as that which occurred in foods of animal origin (Compare Growth Tables 1 and 2). Whether this reduced growth on vegetables was due to nutritional factor(s), pH, competing flora, or the inability of *A. hydrophila* to degrade plant tissue is not known. Psychrotrophic growth of *A. hydrophila* was not entirely unanticipated. Eddy (1960) observed the growth of several environmental and food strains at 1 °C within 7 days. Rouf and Rigney (1971) observed growth of five of ten environmental strains at 5 °C within 14 days. As will be shown later, *A. hydrophila* can readily grow when inoculated into ground pork containing various additives and held at 5 °C. *A. hydrophila* is often found in refrigerated meats held under different conditions (Blickstad and Molin 1983; Enfors *et al.* 1979; Grau *et al.* 1985; Nagel *et al.* 1960; Simard *et al.* 1984). A recent study by Gram *et al.* (1987) has suggested that *A. hydrophila* is a significant spoilage organism of fish held at 5 °C. These data and observations support the basic hypothesis that low temperature alone is not sufficient to prevent the growth of *A. hydrophila* in foods.

Salt and pH

Salt (NaCl) and pH are two traditional means of restricting the growth of foodborne pathogens. Recent studies from this laboratory (Palumbo *et al.* 1985b) have provided data on the NaCl and pH limits for *A. hydrophila*. When *A. hydrophila* was grown in culture media, it was less able to tolerate extremes of NaCl (Fig. 2) or pH (Fig. 3) as temperature was decreased.

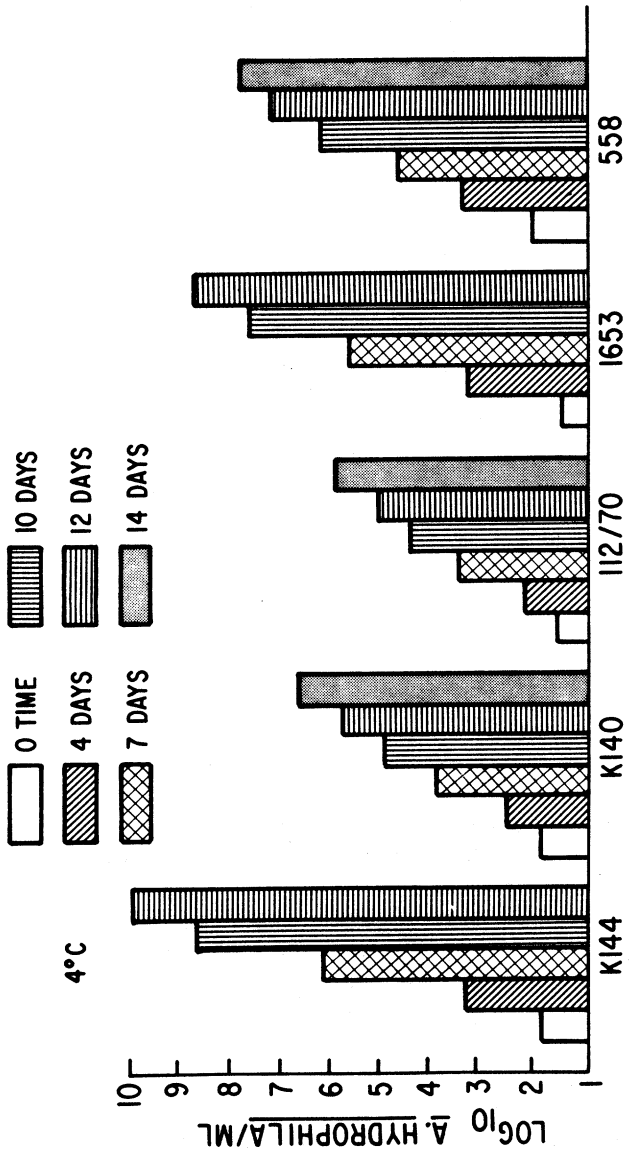

FIG. 1. GROWTH OF FIVE CLINICAL ISOLATES OF *A. HYDROPHILA* IN BHI BROTH AT 4°C, VIABLE COUNT ON NUTRIENT AGAR
(Palumbo *et al.* 1985b).

TABLE 1.
GROWTH OF NATURALLY OCCURRING *A. HYDROPHILA* IN RETAIL FRESH FOODS OF ANIMAL ORIGIN HELD AT 5 °C FOR 0 OR 7 DAYS AND COUNTED ON STARCH AMPICILLIN AGAR

	Log_{10} viable A. hydrophila per gr (or ml) of sample	
food	0 days	7 days
raw shrimp	3.26	6.38
scallops	4.80	7.18
raw shucked oysters	5.70	3.60
croaker	5.20	6.30
bluefish	< 2.00	5.70
fresh sausage	< 2.00	2.40
raw milk	< 1.34	4.70
chicken	3.23	5.70
chicken liver drip	4.45	7.00
ground beef	4.65	5.70
ground lamb	2.78	6.00

Adapted from Palumbo, *et al.* 1985a.

When the growth of *A. hydrophila* K144 was examined in ground pork having different pH values and NaCl levels, it was found that small differences in pH can impact greatly on the microorganism's ability to tolerate NaCl (Palumbo 1988). For example, *A. hydrophila* was completely inhibited by 2% NaCl (3.02% brine) at pH 5.9 (Fig. 4, top), while 3% NaCl (4.72% brine) was needed at pH 6.1 (Fig. 4, bottom). It should be noted that high salt levels at pH values of 6.1 and above, while restricting the growth of *A. hydrophila*, did not cause the organism to die off (See Fig. 2).

The organism seems particularly sensitive to pH values below 6.0. This can be seen in the control of Fig. 4 where, in the absence of salt, cells grew more slowly and to a lower population at pH 5.9 (top) than at pH 6.1 (bottom). The impact of pH was also observed in conjunction with the addition of 1% glucose to the ground pork (Palumbo 1988) (Fig. 5). Even though lactic acid bacteria did not exceed 10^7/g, the pH declined from 6.0 to 5.1 after 20 days of storage. The decline in *A. hydrophila* cfu/g paralleled the decline in pH. This pH effect may

TABLE 2.
NUMBER (log_{10}) OF NATURALLY OCCURRING *AEROMONAS SP.* IN RETAIL FRESH VEGETABLES HELD AT 5°C FOR 0, 7 AND 14 DAYS AND ENUMERATED ON STARCH AMPICILLIN AGAR

vegetable	log_{10} cfu/gr		
	0 days	7 days	14 days
broccoli	< 2.00	2.74	5.76
celery	< 2.00	2.74	3.63
alfalfa sprouts	4.36	3.38	5.49
parsley	< 2.00	3.88	5.79
spinach	3.71	3.08	4.57
endive	< 2.00	2.00	2.18
romaine	2.00	2.00	2.00
kale	< 2.00	2.30	5.45
escarole	< 2.00	2.00	3.04
red leaf lettuce	3.20	2.88	3.66

Adapted from Callister and Agger 1987.

be the explanation for the decline in the number of viable *A. hydrophila* sometimes observed with refrigerated storage of raw shucked oysters (Table 1). Oysters have relatively high levels of glucose which can be fermented by lactic acid bacteria and lower pH values.

The level of NaCl found in fresh sausage may be an explanation for the relative lack of growth of *A. hydrophila* in these products (Table 1). Fresh sausages usually contain 1.5 to 2% NaCl (Kramlich *et al.* 1973) and when converted to % brine, this NaCl level is more than sufficient to inhibit *A. hydrophila*. Thus *A. hydrophila* is not likely to be an organism of concern in any food which has a pH below 6 or a brine level of 3% or greater.

Competing Flora

Pathogens are traditionally viewed as being poor competitors in the presence of indigenous food microflora. To study the influence of background microflora on *A. hydrophila*, we irradiated some ground pork at 300 krads (at 2°C) to reduce the background microflora of the pork. This ground pork was then inoculted with *A. hydrophila* K144 and the viable count of *A. hydrophila* was

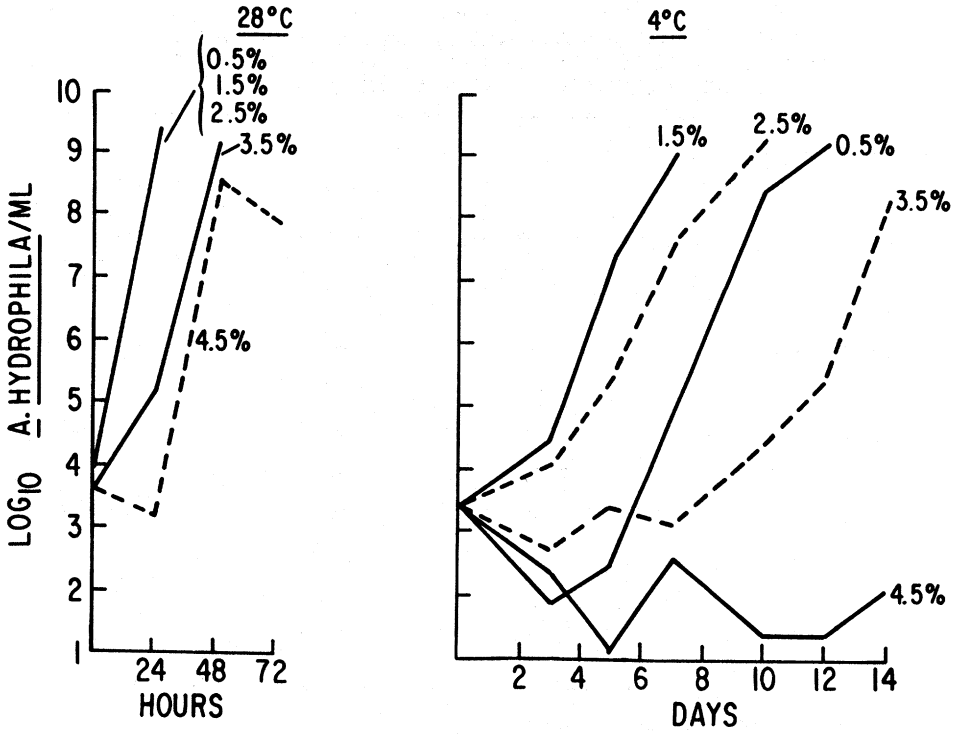

FIG. 2. EFFECT OF NaCl LEVEL AND TEMPERATURE (28 and 5 °C) ON THE GROWTH OF *A. HYDROPHILA* K144 IN BHI BROTH (Palumbo *et al.* 1985b).

followed on starch ampicillin agar. The data in Fig. 6 show that *A. hydrophila* grew to higher levels in the irradiated pork compared to the control. This improved growth was observed in the vacuum pack as well as the aerobic pack (Palumbo 1988). During this time, the background microflora (total and lactic acid bacteria count, Rogosa SL agar) increased in the irradiated samples to at least 10^8/g total count (on APT agar) and 10^7/g lactic acid bacteria, although the counts in the nonirradiated samples tended to be higher.

This response of *A. hydrophila* to the "normal" microflora of ground pork is similar to the response observed in *Yersinia enterocolitica*. Schiemann and Olson (1984) observed an inhibition of *Y. enterocolitica* by Gram negative microflora at 32 °C and found that this inhibition was lessened by incubation at lower temperature. Stern *et al.* (1980) observed that *Y. enterocolitica* could grow in milk at refrigeration temperatures, but it was a poor competitor with common spoilage organisms. Fukushima and Gomyoda (1986) observed an antagonism between *Y. enterocolitica* and the microflora of ground pork held at

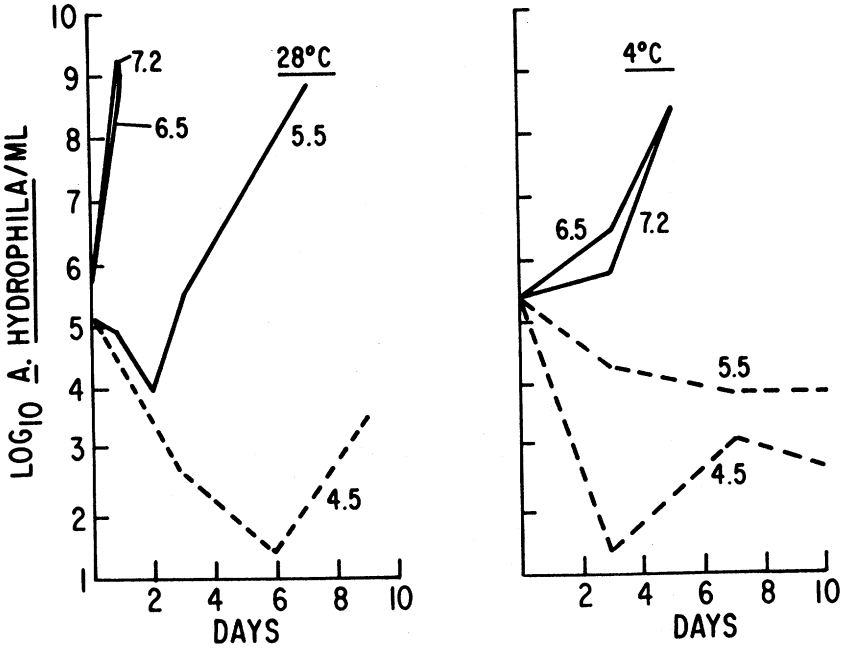

FIG. 3. EFFECT OF pH AND TEMPERATURE ON THE GROWTH OF *A. HYDROPHILA* K144 IN BHI BROTH
(Palumbo *et al.* 1985b).

6 °C. While not completely inhibiting the growth of *A. hydrophila*, the background microflora does limit the maximum attainable population. The effect of competing microflora may be a partial explanation for the relatively low numbers of *A. hydrophila* observed in certain food samples after one week's storage at 5 °C (Table 1).

Irradiation

Though radiation is approved for only a few foods, it possesses the potential for the control of many organisms in numerous products. Recent studies indicated that *A. hydrophila* is relatively radiation-sensitive, with radiation D values in bluefish and ground beef ranging from 14 to 19 Krads at 2 °C (Palumbo *et al.* 1986). *A. hydrophila* responded linearly to increasing doses of radition. This implies the absence of radiation resistant subpopulations.

D-values established for *A. hydrophila* are comparable with those for *Y. enterocolitica* and *Campylobacter jejuni* (Tarkowski *et al.* 1984). However, Salmonellae tend to be more radiation resistant, with reported D values of 55 to 78 Krads (Tarkowski *et al.* 1984). Since the observed radiation resistance of *A.*

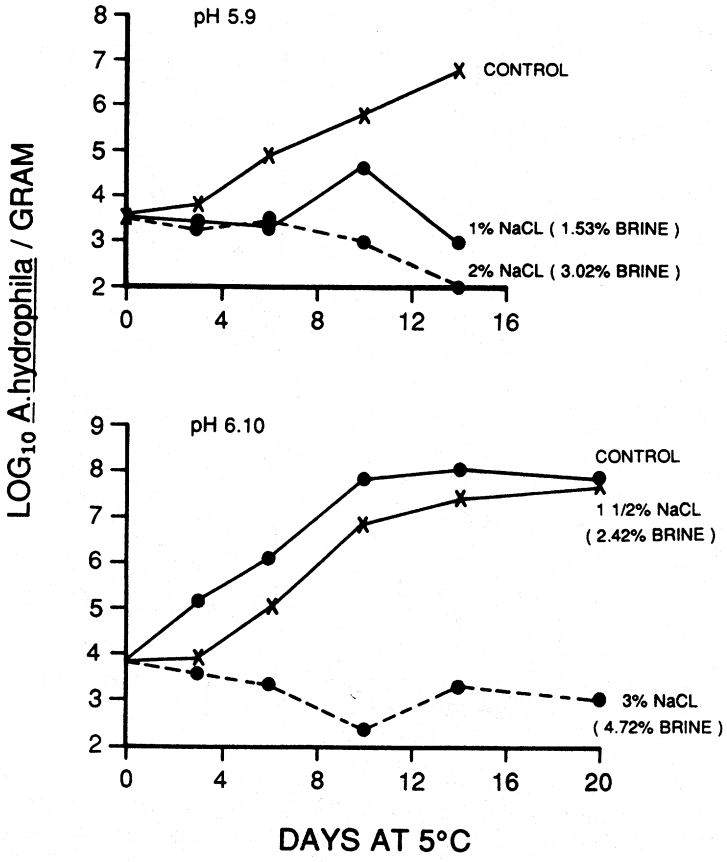

FIG. 4. EFFECT OF NaCl LEVEL (% BRINE) AT pH 5.9 (TOP) AND 6.1 (BOTTOM) ON THE GROWTH OF *A. HYDROPHILA* K144 IN GROUND PORK HELD AT 5°C, AS DETERMINED BY cfu/g ON STARCH AMPICILLIN AGAR

hydrophila is generally similar to other Gram negative bacteria (Ingram and Farkas 1977), the higher reported values for Salmonella make Salmonella appear to be atypical.

The recent food survey of retail fresh foods of animal origin (Palumbo *et al.* 1985a) indicated that the maximum count of *A. hydrophila* at the time of purchase was 1.9×10^5/g in a sample of cod fillet. Based on the data of Palumbo *et al.* 1986, a dose of 150 Krads could be suggested for elimination of *A. hydrophila* in fresh fish and seafood, red meat, and poultry.

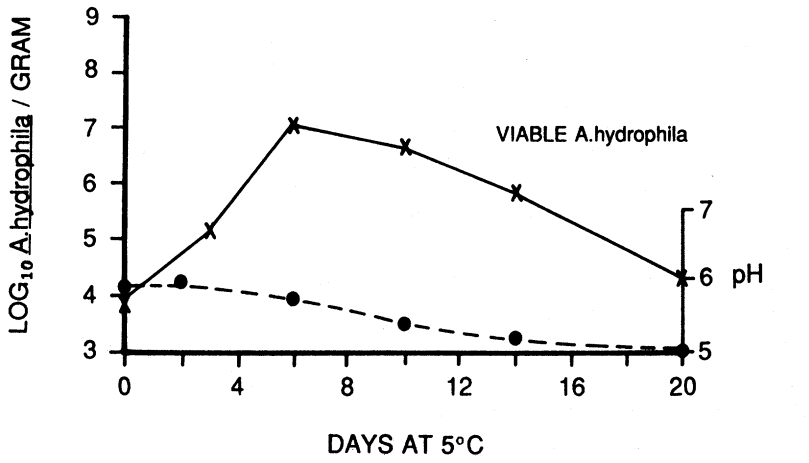

FIG. 5. EFFECT OF 1% GLUCOSE ON THE pH (DASHED LINE) AND *A. HYDROPHILA* K144 VIABILITY (SOLID LINE) IN GROUND PORK HELD AT 5°C, AS DETERMINED BY COUNTING ON STARCH AMPICILLIN AGAR

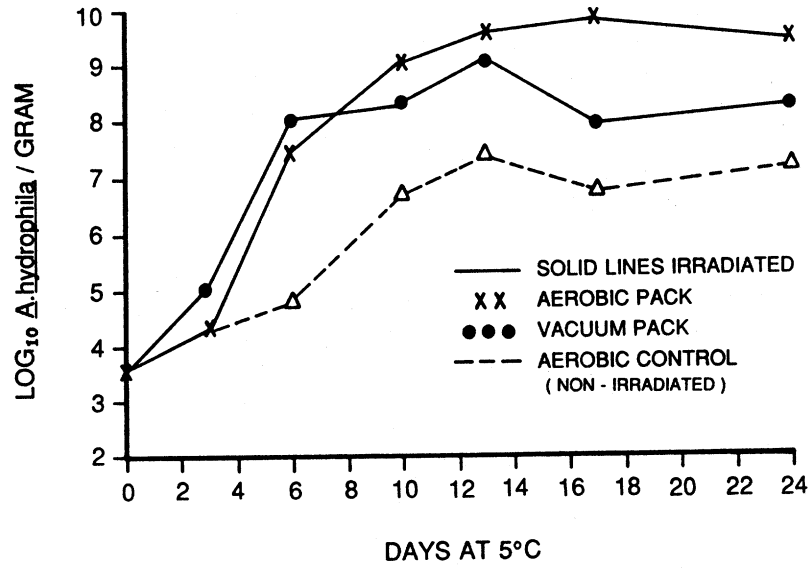

FIG. 6. EFFECT OF BACKGROUND MICROFLORA AND PACKAGING ON THE NUMBER OF *A. HYDROPHILA* K144 IN GROUND PORK HELD AT 5°C DETERMINED AS VIABLE COUNTS ON STARCH AMPICILLIN AGAR

Heating

A recent study (Palumbo *et al.* 1987) examined factors affecting thermal resistance of *A. hydrophila*. Thermal resistance data for *A. hydrophila* indicated a biphasic response at 48 °C and above (Palumbo *et al.* 1987) which is in contrast to our observations on radiation killing of this organism. This biphasic response indicated that the cultures of *A. hydrophila* contained a heat resistant subpopulation. D values for the initial linear phase of the killing curve are presented in Table 3. Most variables caused relatively small differences in the D values, and statistical analyses revealed no distinct pattern among variables. Overall, there appeared to be no difference between clinical (K144, and BW37) and food isolates (B2-10 and F6-10) in their response to heat. However, growth of the cultures at low temperatures, 5 °C, which simulates food holding conditions, yielded cells which were more heat sensitive (lower D values). Further, except for the BA2 culture, heating in raw milk also gave lower D values.

Chlorine and Other Disinfectants

A. hydrophila occurs widely in nature in various water supplies, both chlorinated and raw, (Burke *et al.* 1984a, b; Hazen *et al.* 1978; Le Chevallier *et al.* 1980, 1982; Slade *et al.* 1986). This undoubtedly is the origin of the species name- *hydrophila* 'water-loving'. Its presence in foods such as poultry and vegetables may be due to the water used in their processing. Its presence in chlorinated water suggests that *A. hydrophila* is resistant to chlorine, at least to the levels normally used in the treatment of water for drinking purposes. Cattabiani (1986) studied the susceptibility of *A. hydrophila* to disinfectants such as chlorine (hypochlorite), iodophor, quarternary ammonium compound, glutaraldehyde, and chlorophenol. Representative data are presented in Table 4. As can be seen from Table 4, the levels of chlorine which are lethal to *A. hydrophila* are well below those used in cooling water treatments and drinking water purification. Thus, the proper use of these compounds especially chlorine during food plant sanitation should effectively control *A. hydrophila* con food plant equipment. As indicated above, *A. hydrophila* is often isolated from chlorinated drinking water. This isolation may represent post treatment recontamination, the presence of very large numbers of organisms, or the presence of organic matter which will inactivate the added chlorine.

Freezing and Freeze-Drying

There have been very few studies on the stability/survival of *A. hydrophila* to freezing or freeze-drying. Unpublished observation from our laboratory has indicated that only 20% of *A. hydrophila* isolates survived freeze-drying in skim milk. However, Popoff (1984) has indicated that freeze-drying is a good method of preserving *A. hydrophila* cultures.

TABLE 3.
INFLUENCE OF GROWTH TEMPERATURE, HEATING TEMPERATURE, AND HEATING MENSTRUM ON THE THERMAL RESISTANCE OF CLINICAL (K144, BA2, & BW37) AND FOOD (B2-10 & F6-10) ISOLATES OF *A. HYDROPHILA*

		D values (min)				
		Isolates				
growth temp °C	heating temp °C	K144	BA2 (in saline)	BW37	B2-10	F6-10
28	45	13.84*	29.54	25.97	12.01	13.47
28	48	5.54	5.50	6.64	3.49	4.64
28	51	1.23	2.34	1.80	1.76	2.22
	Z value, °C	5.71	5.45	5.22	6.98	7.69
5	48	3.39	3.50	3.09	2.84	2.75
		in raw milk				
28	48	4.10	6.23	4.73	3.20	3.31

*from initial linear portion of inactivation plot.
Adapted from Palumbo *et al.* 1987.

Abeyta *et al.* (1986) indirectly studied the survival of *A. hydrophila* during frozen storage. They sampled oysters which had been harvested from a growing area associated with foodborne illness and then frozen at −72 °C for 1½ years. They determined an MPN of 9.3 *A. hydrophila* per 100 g of oysters. They found that trypticase soy broth + ampicillin as an enrichment gave the highest recoveries and that MacConkey's agar also gave better recoveries than other media more selective for *A. hydrophila*. While they had no way of determining how the viable numbers changed during frozen storage and what effect other freezing conditions might have had, the organism did maintain viability and the isolated from the oysters were still positive for virulence-associated factors such as hemolysin, suckling mouse, and Y-1 adrenal cell assays.

TABLE 4.
SENSITIVITY OF FOUR STRAINS OF *A. HYDROPHILA* TO DISINFECTANTS

compound	concentration	time of exposure at 25°C, min		
		1	5	10
sodium hypochlorite	5 ppm	-*	-	-
	2.5 ppm	+	-	-
	1.25 ppm	+	3+/1-	1+/3-
	0.625 ppm	+	3+/1-	1+/3-
	0.31 ppm	+	+	+
quaternary ammonium compd	1:12,500	-	-	-
	1:25,000	3+/1-	2+/2-	-
	1:50,000	+	3+/1-	2+/2-
	1:100,000	+	+	+
iodoform	10 ppm	3+/1-	1+/3-	-
	5 ppm	+	+	+
	1 ppm	+	+	+
2-chlorophenol	0.2%	-	-	-
	0.1%	+	-	-
	0.05%	+	2+/2-	-
glutaraldehyde	0.125%	-	-	-
	0.0625%	3+/1-	-	-
	0.031%	+	-	-

*−sensitive: reduction of four or more log cycles in viable count
+resistant
Adapted from Cattabiani (1986).

SUMMARY AND CONCLUSIONS

A. hydrophila occurs widely in retail animal and plant foods. Since it is psychrotrophic, traditional refrigeration (holding foods at 5 °C) can not be used restrict its growth in foods. Naturally occurring strains and clinical isolates can grow competitively both in culture systems and foods held at 5 °C. It is readily destroyed by either heat (D-values at 48 °C = 3.5–6.7 min; Z-values 5.2–7.8 °C) or irradiation (D-values at 2 °C = 14 to 19 Krads) and is sensitive to pH values below 6.0 and NaCl levels (% brine) of *ca.* 3.5%. It is susceptible to commonly used food plant sanitizers and will withstand extended frozen storage in certain foods. Except for its psychrotrophic nature, the behavior and control of *A. hydrophila* make it appear similar to other Gram negative foodborne pathogens.

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